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### STERILITY IN THE NORTH AMERICAN LAKE CRESS *NEOBECKIA AQUATICA* (BRASSICACEAE): INFERENCES FROM CHROMOSOME NUMBER

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#### ABSTRACT

Sterility of lake cress results from uncertain factors and may be a significant element in the decline of populations over the past century. The inability of lake cress (*Neobeckia aquatica*) to produce viable seeds restricts its dispersal to vegetative fragments which are transported less effectively over long distances. We obtained mitotic counts of  $2n = 24$  for individuals from seven populations of lake cress, a species for which the chromosome number was unreported previously. In context of chromosome number distribution in the Brassicaceae based on literature reports for 192 mustard genera, the base number of tribe *Arabideae* (in which lake cress is placed) and of all genera presumed to be closely related to lake cress is  $x = 8$ . The presence of 24 chromosomes indicates that lake cress is a triploid derived from an  $x = 8$  chromosomal series. Highly sterile triploid hybrids ( $2n = 3x = 24$ ) have also been reported in several genera related to lake cress. The extremely well-developed system of vegetative reproduction in lake cress may partially compensate for its sexual sterility. The discovery that lake cress is triploid offers a specific explanation for its sterility and discloses special considerations for the conservation of this rare species.

Key Words: Brassicaceae, *Arabideae*, *Neobeckia*, triploid, conservation

#### INTRODUCTION

The monotypic North American lake cress, *Neobeckia aquatica* (Eaton) Greene (Figure 1), is distinguished as one of few truly aquatic species in the mustard family, Brassicaceae (Cook, 1990). Lake cress is also known for its heterophylly, extreme range of vegetative polymorphism, and remarkable ability to regenerate





Figure 1. A specimen of the aquatic mustard *Neobeckia aquatica* showing dissected submersed foliage, entire emerged foliage, and racemose inflorescence. Although fruits form occasionally in this species, they typically (as in this specimen) lack seeds. Drawn from *Bryson 8865* (KNK). Bar = 1 cm.



from minute fragments of roots, stems and leaves (Foerste, 1889; La Rue, 1943; Mac Dougal, 1914). Although lake cress has been assigned variously to the genera *Armoracia*, *Nasturtium*, and *Rorippa* (Al-Shehbaz and Bates, 1987), a recent molecular systematic study supports the taxonomic recognition of *Neobeckia* as a monotypic sister genus to *Rorippa* (Les, 1994).

Once widely distributed in eastern North America, lake cress has become rare as a result of significant population losses throughout its former range, particularly in the central portion (Stuckey, 1987; Les, 1994). More detailed distributional information has been summarized in Les (1994). Conservation of lake cress is presently of concern in several states with categories of imperilment including rare (New York; Mitchell, 1986), threatened (Vermont; Crow et al., 1981), and endangered (New Jersey; NJDEP, 1991). Reasons for its rarity are not wholly evident although several explanations have been offered.

Habitat destruction or degradation are often cited as factors contributing to the present rarity of lake cress (e.g., La Rue, 1943; Myers and Henry, 1976; Stuckey, 1987; Swink, 1969). Stuckey (1987) attributed the disappearance of the species to turbidity and chemical pollution, observing that the greatest loss of lake cress populations has occurred in highly agricultural or industrialized regions. However, despite the fact that many extant lake cress populations occur in fairly pristine habitats, we have observed that populations can also thrive in substantially polluted sites (Les, pers. obs.).

Although habitat loss surely is at least partly responsible for the decline of lake cress, other factors must be considered. Lake cress appears to flourish locally and can become abundant once established (La Rue, 1943; Muenscher, 1930; Les, pers. obs.), yet the species has never become common (Al-Shehbaz and Bates, 1987). The local abundance of lake cress has been ascribed to its efficient vegetative reproduction (Pringle, 1879; La Rue, 1943) and tolerance to a wide range of environmental conditions owing to its phenotypic plasticity (Mac Dougal, 1914).

Pringle (1879) concluded that seeds provide the principal means of long-distance dispersal for the species; however, lake cress is known to be highly sterile (Foerste, 1881; La Rue, 1943; Mac Dougal, 1914; Muenscher, 1930, 1944; Gleason and Cronquist, 1991; Long and Lakela, 1971; Godfrey and Wooten, 1981; Al-Shehbaz and Bates, 1987; McCormac, 1992; Les, pers. obs.). Al-



though seeds are produced on occasion (Murley, 1951; Rollins, 1993), their viability has never been demonstrated. La Rue (1943) suggested that the rarity of lake cress might be directly related to its poor seed production which reduces the potential for long-distance dispersal.

Rollins (1966) indicated that chromosome numbers often help to delimit problematic genera in the Brassicaceae. Because chromosome counts were previously unreported for *Neobeckia aquatica*, our primary objective was to establish the chromosome number for assisting with our assessment of its systematic relationship in the mustard family. We hoped that this basic information might shed light on the phylogenetic relationships of lake cress by indicating the specific ploidy level and chromosomal series from which it is derived. In addition, the association of sterility and high polyploidy in some mustards (e.g., in *Dentaria* by Montgomery, 1955), further warranted a cytological examination of lake cress. An explanation for sterility in lake cress is wanting (Rollins, 1993), in spite of the fact that such information could provide important insights to facilitate its recovery and conservation.

#### MATERIALS AND METHODS

To minimize impact on established plants, only detached lake cress leaves or shoot fragments were collected from four populations in Vermont during the summer of 1993 (permit obtained from Vermont Department of Fish and Wildlife). Ronald L. Stuckey and C. B. Hellquist provided us with living lake cress specimens from Ohio and Michigan, respectively. Robert W. Freckmann provided us with fresh lake cress leaves removed from plants collected in Wisconsin from which we regenerated complete plants (Table 1). Adventitious roots were then obtained by removing some of the leaves from the greenhouse plants and floating them in tap water until rooting plantlets regenerated (1–3 weeks).

Mitotic figures were obtained from squashes of newly emerged adventitious root tip cells following methods employed by Bernardello and Anderson (1990). Root tip sections 1–2 cm long were pretreated at room temperature for two hr in saturated aqueous paradichlorobenzene, then rinsed and fixed at room temperature for 18 hr in a 3:1 solution (v/v) of ethanol:acetic acid. Fixed



Table 1. Chromosome counts obtained for populations of *Neobeckia aquatica* from Michigan, Ohio, Vermont, and Wisconsin, USA.

Locality	Voucher	2n Counts Observed
<b>MICHIGAN</b>		
Cheboygan Co. Mullett Lake, Pigeon River Marsh	<i>Hellquist 15542</i> (NASC)	23, 24, 25
<b>OHIO</b>		
Franklin Co. Hoover Reservoir	<i>Stuckey s.n.</i> (CONN)	23, 23/24, 23/24, 24, 24, 25
<b>VERMONT</b>		
Addison Co. Lake Champlain, Catfish Bay	<i>Les s.n.</i> (CONN)	23, 23/25, 24, 24, 26
East Creek	<i>Les s.n.</i> (CONN)	23, 24, 24, 24, 24, 24
Shoreham, Lemon Fair River	<i>Les s.n.</i> (CONN)	24, 24, 25, 25, 25
Grand Isle Co. Lake Champlain, Isle La Motte	<i>Les s.n.</i> (CONN)	24, 24/25
<b>WISCONSIN</b>		
Marinette Co. Peshtigo Flowage	<i>Freckman s.n.</i> (CONN)	23, 24, 24, 25

root tips were stained in alcoholic hydrochloric acid-carmin (Snow, 1963) for five d. Stained root tips were placed in an aqueous 70% acetic acid solution for 30 min, then macerated, lightly heated, and squashed in a drop of 70% acetic acid. Squashes were studied with phase contrast and bright field optics using Olympus and Zeiss microscopes (the latter equipped with a 63× 1.4 NA Planapochromatic objective).

A minimum of 10 root tips/population furnished the two to six mitotic cells selected from each population for chromosome counts. Slides were made permanent with Euparal (Bradley, 1948) and cells were photographed with a Zeiss Universal microscope and Kodak Technical Pan film.

Chromosome numbers were obtained from the literature for 192 genera in 17 of the 19 tribes recognized by Schulz (1936). The complete compilation is available from the authors on request. As Manton (1932) did with "fundamental numbers (f),"



we selected a reasonable ancestral chromosome number ( $x$ ) for each genus, and from these, we inferred an ancestral number for each of the tribes. These determinations took into account discussions by Dvorák (1971), Harberd (1976), Manton (1932), Mulligan (1964, 1965, 1966), Rollins (1963, 1966), Rollins and Rüdtenberg (1971, 1977, 1979) and Rollins and Shaw (1973).

To obtain a phylogenetic perspective, we mapped the inferred base numbers on a phylogenetic diagram of the cruciferous tribes derived from the evolutionary "tree" published by Schulz (1936). We also compared base numbers of aquatic genera in tribe *Arabideae* that appear closely related to lake cress in a cladogram constructed from DNA sequence data (Les, 1994). The placement of lake cress in tribe *Arabideae* (Schulz, 1936) is supported by its close relationship to *Armoracia*, *Cardamine*, *Nasturtium*, and *Rorippa* (Les, 1994). Schulz included all of these genera in tribe *Arabideae* except for *Armoracia* which he placed within tribe *Drabeae*.

#### RESULTS

Examination of lake cress populations revealed  $2n$  counts of 23–26 chromosomes (Figure 2, Table 1). In some instances, the small size of chromosomes (0.6–0.8  $\mu$ ) and presence of unidentified stained particles in the vicinity of nucleolar organizer regions, made counts difficult. The shape and appearance of the extra-chromosomal particles were used to differentiate them from neighboring chromosomes. We consistently detected what appeared to be one pair of chromosomes with small 'satellite' regions in most preparations; however, inadequate resolution made this observation impossible to verify.

We observed cells of two types. Larger cells showed chromosomes dispersed over a lightly stained cytoplasm; smaller cells contained more densely arranged chromosomes against both dark and lightly stained cytoplasm. Chromosomes in smaller cells were generally easier to count because they lay more in a single plane than those of larger cells.

Three greenhouse plants flowered during Fall, 1995, but we were unable to obtain satisfactory preparations for meiotic counts. There were six fruits produced on these plants, but complete ovule abortion rendered them seedless in every instance.



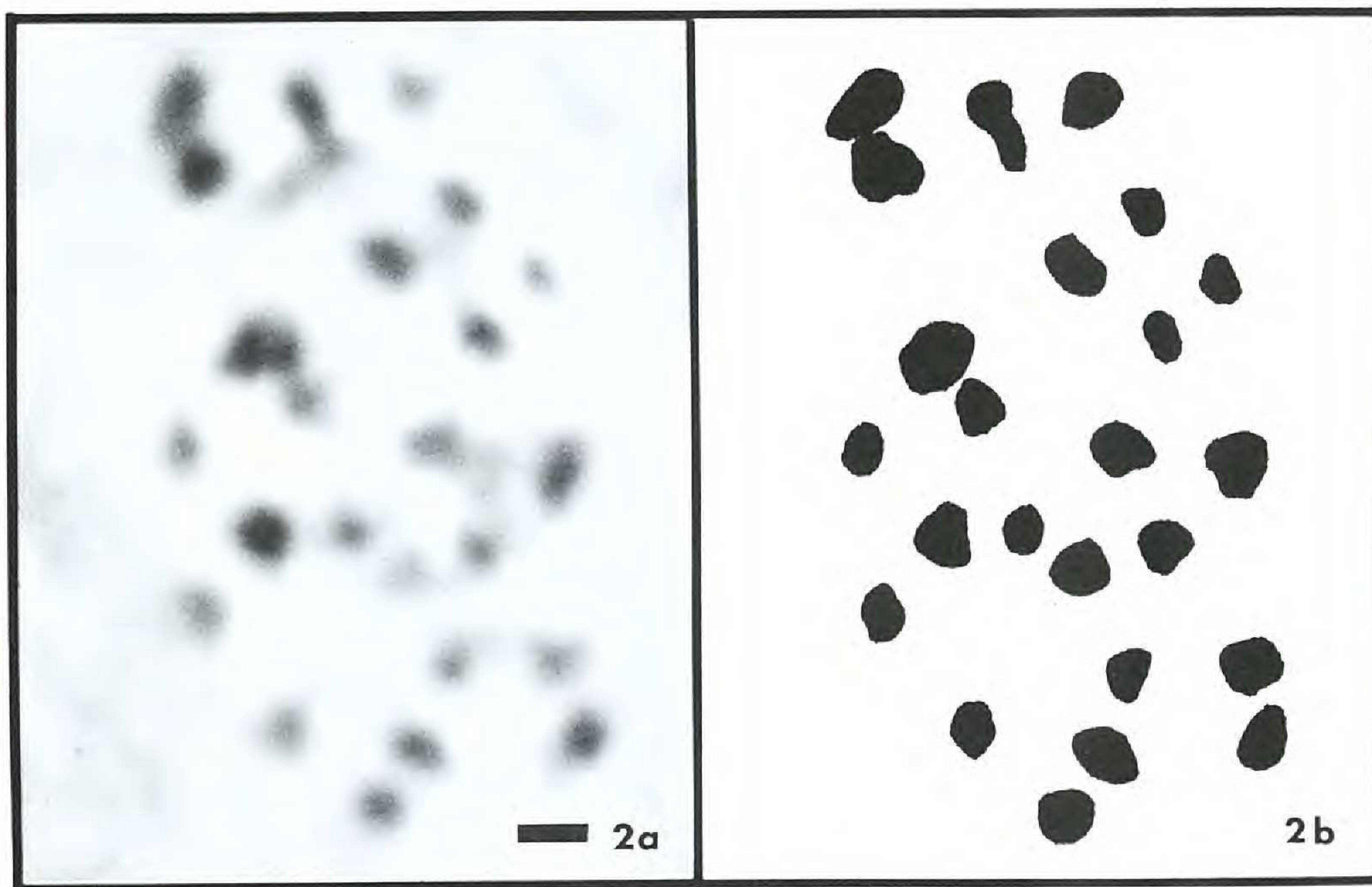


Figure 2. Example of mitosis in adventitious root cells of *Neobeckia aquatica* (Shoreham, Vermont locality). a: Micrograph showing characteristically small metaphase chromosomes. b: Interpretation of micrograph indicates the count  $2n = 24$  chromosomes. Bar = 1  $\mu\text{m}$ .

Our conclusions of tribal base numbers in Brassicaceae (Figure 3a) corresponded well with those proposed by Manton (1932), Mulligan (1964, 1965, 1966), Rollins (1966), and Rollins and Rudenberg (1971, 1977, 1979). In the context of intertribal relationships proposed by Schulz (1936), a general trend toward reduced base numbers is observed from the putatively primitive to the more advanced tribes of the Brassicaceae (Figure 3a).

In 18 genera of tribe *Arabideae* with available counts, 12 (67%) have a likely base number of  $x = 8$  and 4 (22%) a base number of  $x = 7$ . The base number of two genera (*Guillenia* and *Leavenworthia*) could not be reasonably determined. A base number of  $x = 8$  characterizes all genera (*Armoracia*, *Cardamine*, *Nasturtium*, *Rorippa*) putatively allied with lake cress (Figure 3b).

#### DISCUSSION

Although intraspecific aneuploidy is prevalent among aquatic angiosperms (Les and Philbrick, 1993), variation in our reported counts for lake cress (Table 1) reflects the uncertainty of some



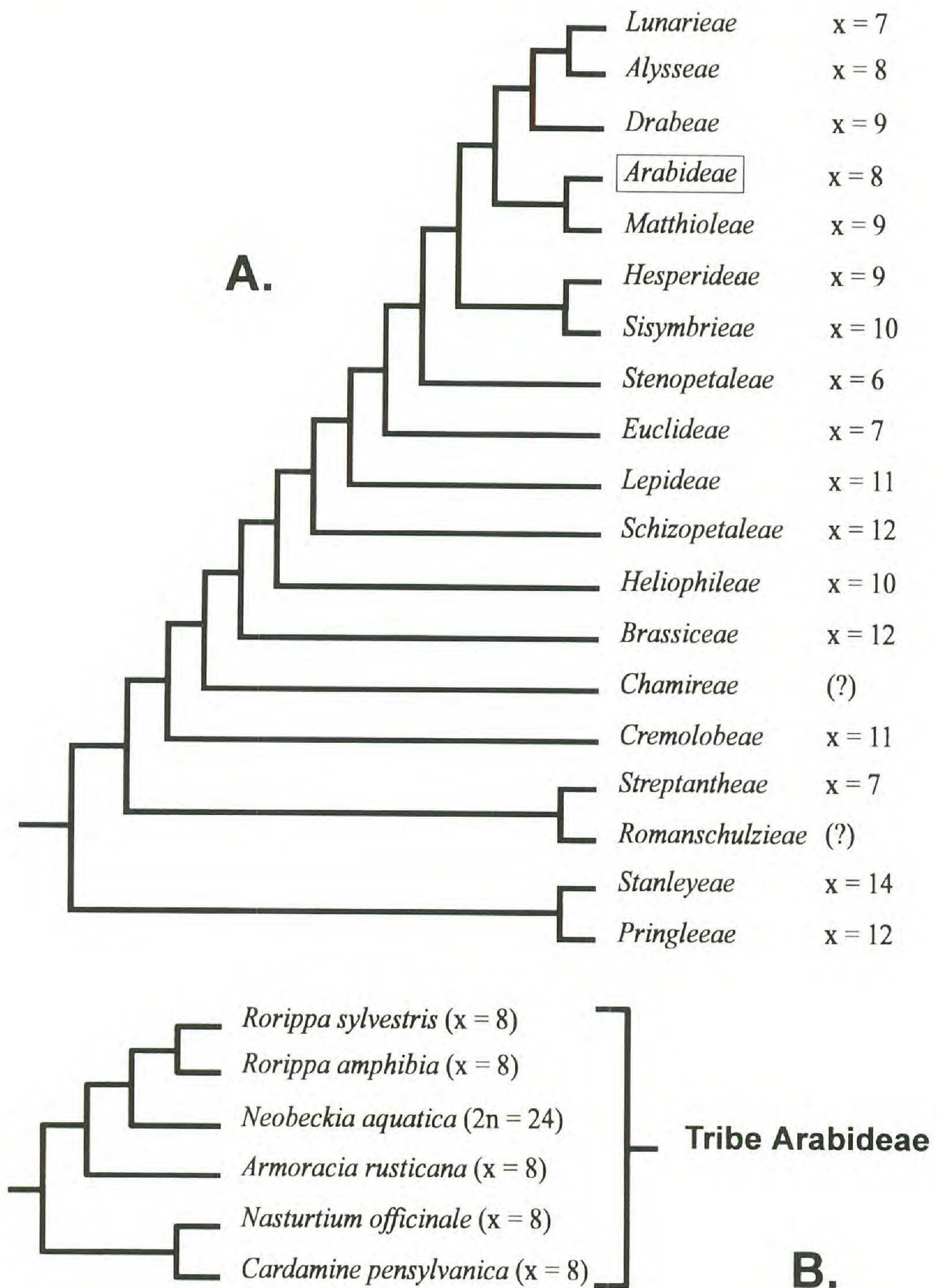


Figure 3. Putative phylogenetic relationships of mustards. A: Phylogenetic scheme of tribal interrelationships (redrawn from Schulz, 1936) and base chromosome numbers deduced from literature reports (see text). A box identifies the tribe (*Arabideae*) to which lake cress is assigned. In this interpretation, a general decrease in the base number is apparent from the putatively primitive to more advanced tribes. B: Phylogenetic relationships of aquatic mustard genera in tribe *Arabideae* (from Les, 1994) with deduced base chromosome numbers and the  $2n$



counts and does not necessarily indicate the existence of discrete numbers for the species. We are convinced that the actual chromosome number of the species is  $2n = 24$ ; however, in several preparations, it was difficult to clearly view all chromosomes or to positively differentiate chromosomes from anomalous inclusions (see Results). In several instances, this 'variation' was observed among cells from the same individual and from different individuals of the same population. Because of the clonal growth of this species, and lack of sexual reproduction, our replicate counts were not of individual genets but of clonally derived ramets.

Stuckey (1972) hypothesized that the genera *Armoracia*, *Cardamine*, *Nasturtium*, and *Rorippa* represented the closest relatives of *Neobeckia*, a conclusion consistent with a recent molecular systematic study of the group (Les, 1994). Hayek (1911) placed these genera together not only in the same tribe (*Arabideae*) but in the same subtribe (*Cardamininae*). Literature counts reported for these genera uniformly indicate a base number of  $x = 8$ . A basic number of  $x = 8$  has also been determined for *Dentaria*, a genus sometimes merged with *Cardamine* (Montgomery, 1955). In tribe *Arabideae*, the base number  $x = 8$  is very common with only a few instances of  $x = 7$  or  $x = 6$  (see Harberd, 1976).

Because they are not cladistically based, the phylogenetic relationships of mustard tribes (Figure 3a) proposed by Schulz (1936) must be interpreted conservatively. However, we are unaware of any more recent studies of intertribal relationships in the mustard family that are as comprehensive. Molecular systematic studies of *Arabidopsis* by Price et al. (1994) included representatives of only 5–6 mustard tribes. Although topologies of the molecular cladograms differed in some details from the phylogenetic tree of Schulz (1936), the relatively derived position of tribe *Arabideae* and the relatively basal position of tribes with higher basic numbers were consistently indicated. Thus, it is reasonable to conclude that chromosomal series with basic numbers less than twelve are probably derived by descending aneuploidy in advanced tribes such as *Arabideae*. Genera of tribe *Arabideae* that are closely

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number obtained for *Neobeckia*. A base chromosome number of  $x = 8$  characterizes tribe *Arabideae* and the aquatic genera related to *Neobeckia*.



related to *Neobeckia* (Les, 1994) share the basic number of  $x = 8$  (Figure 3b). Given that the most likely base number of tribe *Arabideae* is  $x = 8$ , the  $2n = 24$  chromosome number of *Neobeckia aquatica* indicates that the species is triploid, at least in the populations studied.

The number  $2n = 24$  is rare in tribe *Arabideae* and is indeed associated with triploid hybrids. In *Rorippa*, spontaneous triploids ( $2n = 24$ ) have resulted from hybridization between two tetraploids ( $2n = 32$ ), e.g., *R. amphibia* (L.) Besser and *R. palustris* (L.) Besser (Howard, 1947; Stace, 1975). The triploid hybrids are sterile with 7–8 bivalents and 8–10 univalents, whereas tetraploid hybrids are fertile. Mulligan and Porsild (1968) reported a natural triploid ( $2n = 24$ ) hybrid between diploid ( $2n = 16$ ) *R. barbareaefolia* (DC.) Kitagawa and tetraploid ( $2n = 32$ ) *R. palustris* (= *R. islandica* (Oeder) Borbás). The triploids did not produce seed and had only 2% viable pollen. Triploid ( $2n = 24$ ) hybrids also have been produced artificially in crosses between diploid ( $2n = 16$ ) *R. austriaca* (Crantz) Besser and tetraploid ( $2n = 32$ ) *R. sylvestris* (L.) Besser (Jonsell, 1968; Javurková-Kratochvílová and Tomsovic, 1972). In one case, only 14% of the seeds produced from this cross germinated; however, none of the plants ever reached the flowering stage (Jonsell, 1968). Sterile triploid hybrids ( $2n = 24$ ) have also resulted from crosses between diploid ( $2n = 16$ ) *R. austriaca* and tetraploid ( $2n = 32$ ) *R. amphibia* (Javurková-Kratochvílová and Tomsovic, 1972; Jonsell, 1975).

In *Cardamine*, triploid ( $2n = 24$ ) hybrids are produced in crosses between two diploids ( $2n = 16$ ), *C. rivularis* Schur and *C. amara* L. (Urbanska-Worytkiewicz, 1977). Reproduction of these triploids is mainly vegetative but they are partly fertile with about 2–3% pollen viability (Urbanska-Worytkiewicz, 1977).

To enumerate, the triploid chromosome number of lake cress is indicated by the common occurrence of the  $x = 8$  base number in the tribe *Arabideae*, the universal occurrence of  $x = 8$  among the genera most closely related to lake cress, and the presence of the  $2n = 24$  chromosome number in known triploid hybrids within tribe *Arabideae*. In addition, a significant correlation is the extreme sterility of lake cress, a feature long associated with triploidy in plants (Darlington and Mather, 1949). The relatively low chromosome number of lake cress indicates that sterility is not a consequence of high ploidy level as in the related genus *Dentaria* (Montgomery, 1955).

Sterility in the related horseradish (*Armoracia rusticana* L.) has



been attributed to an interspecific hybrid origin (Weber, 1949), self-incompatibility, and accumulation of deleterious mutations from prolonged vegetative propagation (Stokes, 1955). Self-incompatibility has also been implicated in the low seed production of perennial *Rorippa* species, presumably due to the clonal growth of populations (Jonsell, 1968).

We also considered that sterility of lake cress may be a consequence of self-incompatibility (SI). Poor seed set is an inevitable consequence for clonal, SI species that experience extreme bottlenecks (Les, Reinartz, and Essleman, 1991; Reinartz and Les, 1994). In a survey of the mustard family, Bateman (1955) found that SI species occurred in 11/12 tribes surveyed, including the genera *Armoracia* and *Cardamine*. Self-incompatible species also occur in *Rorippa* (Jonsell, 1968), the most closely related genus to *Neobeckia*. Although self-compatible species have evolved repeatedly in the family, e.g., in *Cardamine*, *Nasturtium* and *Rorippa* (Bateman, 1955; Jonsell, 1968), SI could certainly be expected in *Neobeckia*. Sterility, however, precludes verification of SI in lake cress by experimental crossing studies. Nevertheless, triploidy is more likely to represent the proximate cause of sterility in the species.

The origin of triploidy in lake cress remains uncertain. At this time, we cannot determine whether the species represents an intraspecific hybrid or an interspecific hybrid resulting from a cross between diploids (e.g., *Cardamine*, above), tetraploids (e.g., *Rorippa*, above), or possibly a diploid and tetraploid (e.g., *Rorippa*, above).

Our study has confirmed that the triploid condition exists among different lake cress populations separated in some cases by more than 100 km in the northeastern USA, and in considerably more isolated populations from Michigan, Ohio, and Wisconsin. This indicates that the unusual cytotype is not simply a local, vegetatively propagated abnormality (see Les and Philbrick, 1993) but is widespread and at least characteristic of the northern populations. To our knowledge, the Wisconsin population represents the northwesternmost known station, and the Isle La Motte, Vermont population, the easternmost station for the species. The triploid number from the Ohio plant is also consistent with observations that other Ohio populations of lake cress may flower and fruit quite prolifically, yet apparently produce no viable seed (McCormac, 1992).

We cannot exclude the possibility that diploid or tetraploid



cytotypes of lake cress may exist. Potentially, such individuals should be more fertile, yet seed set could remain scarce in populations due to confounding factors such as self-incompatibility. This is precisely the situation for *Apios americana* Medikus with sterile triploid populations in northern parts of its range, and diploid populations in the southern portion of its range in which seed set remains low due to self-incompatibility (Bruneau and Anderson, 1988). The possible existence of diploid or tetraploid lake cress can only be ascertained by further cytological examination of populations throughout its range. Our attempts to obtain material of this rare species from southern portions of its range, however, have thus far proven to be unsuccessful. A comprehensive survey of lake cress populations is highly recommended because the discovery of fertile plants could significantly influence conservation strategies for the species.

Except in the relatively rare cases of partially fertile triploids (e.g., *Cardamine*; Urbanska-Worytkiewicz, 1977), the sexual reproductive capacity of triploid plants is predictably low. The highly developed system of vegetative reproduction in lake cress provides a means of dispersal and reproduction despite the barrier to sexual reproduction that is imposed by its triploid cytotype. The lack of seed production, however, greatly compromises the ability of the species to disperse beyond local distances. Although shoot fragments of several other aquatic plants can be dispersed considerable distances by flexuous stems that become draped over waterfowl, etc. (Sculthorpe, 1967), the brittle nature of lake cress plants undoubtedly precludes this avenue of dispersal. Leaves of lake cress are the most likely vegetative propagules, and are probably difficult to transport over any significant distance.

Both habitat loss and the low vagility resulting from sterility can be linked to the disappearance of lake cress. In the past when habitats were abundant, the species may have survived adequately despite its poor vagility. Extensive habitat destruction coupled with the inability to effectively disperse may now destine the lake cress to successive population losses and ultimate demise. It is unlikely that vegetative propagules alone can adequately maintain dispersal among the remaining fragmented lake cress habitats. Without intervention, sites that have experienced local extirpations due to population crashes are likely to remain devoid of the species in a progression that may inevitably lead to extinction.



The realization that sterility in lake cress may be due to specific genetic (i.e., chromosomal) factors is important from a conservation standpoint. For example, it has long been known that vegetatively reproducing mustard crops (i.e., horseradish and watercress) can be severely damaged by fungal and viral pathogens (Crisp, 1976). Such threats are particularly serious for clonal plants like lake cress where seed production cannot be relied upon as a means of purging pathogens (Silander, 1985). In addition to the obvious importance of preserving remaining lake cress habitats, the implementation of artificial establishment techniques should be considered as a strategy to overcome the dispersal limitations imposed on this species by both biological and cultural factors.

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